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## SYNTHESIS AND PHYSICOCHEMICAL PROPERTIES OF [19,20-<sup>13</sup>C]-17 $\alpha$ -ETHINYLESTRADIOL

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### SUMMARY

<sup>13</sup>C<sub>2</sub>-17 $\alpha$ -ethinylestradiol (<sup>13</sup>C<sub>2</sub>-EE<sub>2</sub>) was synthesized from estrone and <sup>13</sup>C<sub>2</sub>-C<sub>2</sub>H<sub>2</sub>-gas to measure the metabolic clearance rate and the plasma concentration of 17 $\alpha$ -ethinylestradiol (EE<sub>2</sub>) in tall girls, who are treated with high dosages of this estrogen. Interesting characteristics determined by (i) MS: the molecular ion m/z 298.183 is the only labelled ion in the spectrum, and by (ii) <sup>1</sup>H-NMR  $\delta$ (ppm) 2.96 (dd, <sup>1</sup>J(<sup>13</sup>C-H)=249 Hz, <sup>2</sup>J(<sup>13</sup>C-H)=49.6 Hz; 1H; H<sub>20</sub>) and (iii) <sup>13</sup>C-NMR  $\delta$  88.8 (dd, <sup>1</sup>J(<sup>13</sup>C-<sup>13</sup>C)=166 Hz, <sup>2</sup>J(<sup>13</sup>C-H<sub>20</sub>)=49.8 Hz; C<sub>19</sub>); 80.2 (dd, <sup>1</sup>J(<sup>13</sup>C<sub>17</sub>-<sup>13</sup>C<sub>19</sub>)=73 Hz, <sup>2</sup>J(<sup>13</sup>C<sub>17</sub>-<sup>13</sup>C<sub>20</sub>)=12.3 Hz; C<sub>17</sub>) and 74.6 (dd, <sup>1</sup>J(<sup>13</sup>C-<sup>13</sup>C)=166 Hz, <sup>1</sup>J(<sup>13</sup>C-H)=249 Hz; C<sub>20</sub>) confirm the synthesis and structure of <sup>13</sup>C<sub>2</sub>-EE<sub>2</sub>.

### INTRODUCTION

Administration of estrogens to healthy, (pre)pubertal tall girls will cause a decreased growth rate, accelerated sexual maturation, epiphyseal fusion and, as desired, a reduced final adult height (ref. 1).

In order to keep the potential complications of the estrogen therapy, existing of 2x 50  $\mu$ g 17 $\alpha$ -ethinylestradiol (EE<sub>2</sub>) per os daily, as low as possible it would be necessary to measure carefully the individual concentration time relationship of EE<sub>2</sub> in plasma in relation to growth rate, skeletal maturation and other parameters. For this purpose we decided to synthesize <sup>13</sup>C-labelled 17 $\alpha$ -ethinylestradiol (<sup>13</sup>C<sub>2</sub>-EE<sub>2</sub>) according to the previously published method (ref.2).

In this paper the synthesis and main physicochemical characteristics of <sup>13</sup>C<sub>2</sub>-EE<sub>2</sub> are described.

### METHODS

#### Instrumentation

Centrifugal liquid chromatography was performed on a Hitachi CLC-5 instrument. A rotating disk of 3 mm silicagel 60-G (Merck, Darmstadt, G.) and 10 cm path length was eluted with chloroform (Merck, p.a.) at a rate of 8.3 ml. min<sup>-1</sup> at 480 rpm approximately. Mass spectra were recorded on a

AEI-MS-9 spectrometer. The infrared spectrum was recorded on a Unicam SP-200 spectrophotometer in a KBr pellet. Nuclear magnetic resonance spectra were recorded on a Varian-VXR-300 at 300 MHz for  $^1\text{H}$ -NMR and at 75.48 MHz for  $^{13}\text{C}$ -NMR in  $\text{CD}_3\text{OD}$ .  $^1\text{H}$ -chemical shifts are reported in  $\delta$  units (ppm) relative to TMS and  $^{13}\text{C}$ -chemical shifts relative to  $\text{CD}_3\text{OD}$ .

### Synthesis

Approximately 3 mmole  $^{13}\text{C}_2\text{-C}_2\text{H}_2$  gas (MSD Isotopes) was condensed in a 250 ml three necked flask containing a mixture of 1.5 ml 1.5 N n-Buli/n-hexane and 15 ml distilled THF at  $-115^\circ\text{C}$ . During warming up to  $0^\circ\text{C}$  the mixture was stirred. Then a solution of 1.1 mmole estrone (Aldrich) in 20 ml dry THF was added dropwise and the mixture was stirred during 30 minutes at  $0^\circ\text{C}$  and 15 minutes at  $40^\circ\text{C}$ . After cooling to room temperature a solution of 12 g  $\text{NH}_4\text{Cl}$  in 60 ml  $\text{H}_2\text{O}$  was added dropwise and the resulting mixture was stirred 30 minutes. The product was extracted with  $\text{CHCl}_3$  (2x 100 ml) and the organic layer was dried on  $\text{MgSO}_4$ , filtered and evaporated to dryness to yield 365 mg of crude steroid as a white powder. Spectroscopically pure  $^{13}\text{C}_2\text{-EE}_2$  was obtained after chromatography on a Hitachi CLC-5 chromatograph using  $\text{CHCl}_3$  as the eluent and monitoring at 254 nm. After two runs 0.67 mmole of pure  $^{13}\text{C}_2\text{-EE}_2$  was obtained as a white mass.

### RESULTS

Fig.1 shows the structure and the numbering of the carbon atoms of  $^{13}\text{C}_2\text{-EE}_2$  ( $^{13}\text{C}$  indicated by an asterix).

The mass spectra of  $^{13}\text{C}_2\text{-EE}_2$  (a) and  $\text{EE}_2$  (b) are shown in fig. 2. Apart from very small fragments at  $m/z > 250$  the only labelled fragment is the molecular ion of  $m/z$  298. The exact mass of the molecular ion was measured and calculated to be 298.183 and 298.184, respectively.

Absorbance maxima of  $^{13}\text{C}_2\text{-EE}_2$  in the infrared were recorded at 3350 (s), 2950 (s), 1620 (m), 1510 (m), 1460 (m), 1290 (m), 1240 (m) and 1025 (s)  $\text{cm}^{-1}$ .

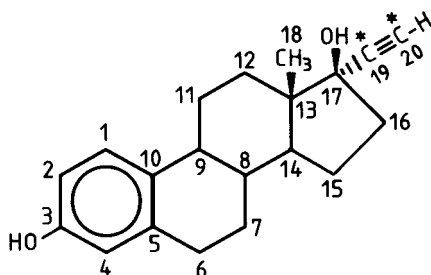


Fig.1. [19,20- $^{13}\text{C}$ ]-17 $\alpha$ -ethinyl-estra-1,3,5-triene-3,17 $\beta$ -diol

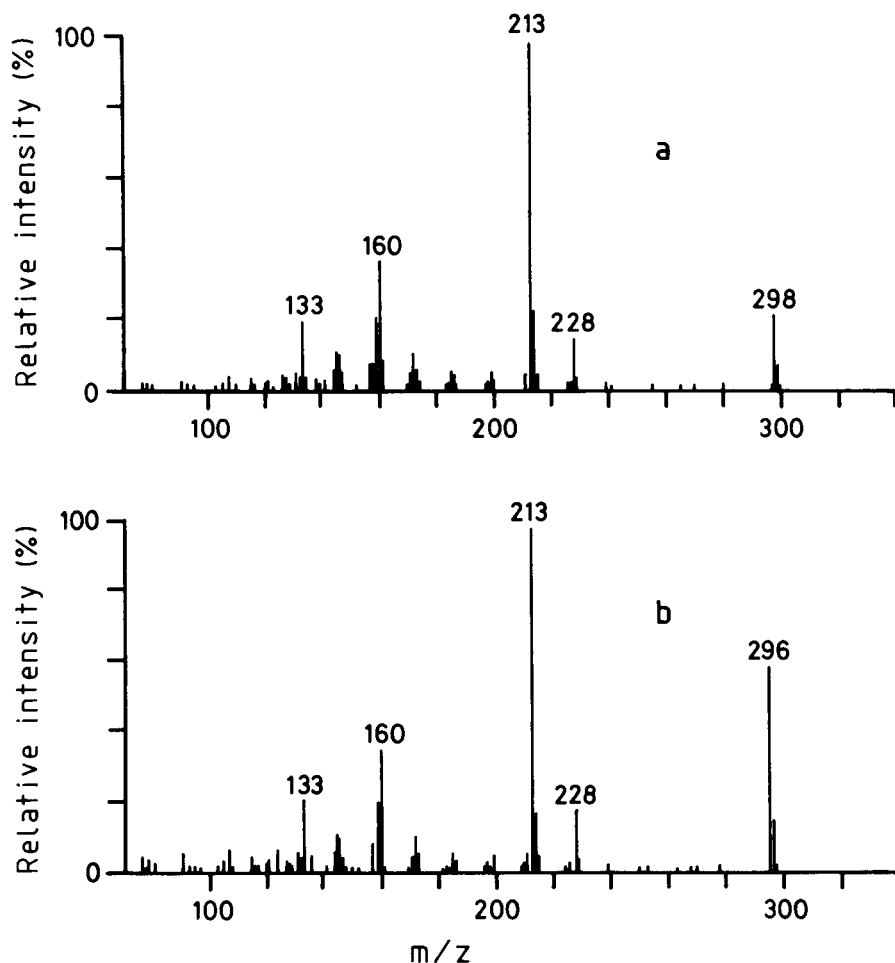


Fig.2. Electron impact mass spectra (70eV) of  $^{13}\text{C}_2\text{-EE}_2$ (a) and  $\text{EE}_2$ (b)

$^1\text{H-NMR}$  of  $^{13}\text{C}_2\text{-EE}_2$  was recorded in  $\text{CD}_3\text{OD}$  at 300 MHz. The chemical shifts are given in  $\delta$  units (ppm) relative to TMS: 7.21(d,  $J=8.4$  Hz;  $1\text{H}; \text{H}_2$ ); 6.72(d,  $J=8.4$  Hz;  $1\text{H}; \text{H}_1$ ); 6.65(s,  $1\text{H}; \text{H}_4$ ); 2.96(dd,  $^1J(^{13}\text{C-H})=249$  Hz,  $^2J(^{13}\text{C-H})=49.6$  Hz;  $1\text{H}; \text{H}_{20}$ ); 2.9(m,  $2\text{H}$ ); 2.45(m,  $2\text{H}$ ); 2.33-1.8(m,  $7\text{H}$ ); 1.65-1.3(m,  $4\text{H}$ ); 1.00(s,  $3\text{H}; \text{H}_8$ ).

$^{13}\text{C-NMR}$  of  $^{13}\text{C}_2\text{-EE}_2$  was recorded in  $\text{CD}_3\text{OD}$  at 75.48 MHz. The chemical shifts are given in  $\delta$  units (ppm) relative to  $\text{CD}_3\text{OD}$ . The assignments of these shifts to the carbon atoms were derived from the APT spectrum and the coupled and uncoupled spectra. The uncoupled spectrum (fig. 3) shows strong resonance at 88.8(dd,  $^1J(^{13}\text{C}-^{13}\text{C})=166$  Hz,  $^2J(^{13}\text{C}-\text{H}_{20})=49.8$  Hz;  $\text{C}_{19}$ ); 74.6(dd,  $^1J(^{13}\text{C}-^{13}\text{C})=166$  Hz,  $^1J(^{13}\text{C-H})=249$  Hz;  $\text{C}_{20}$ ),

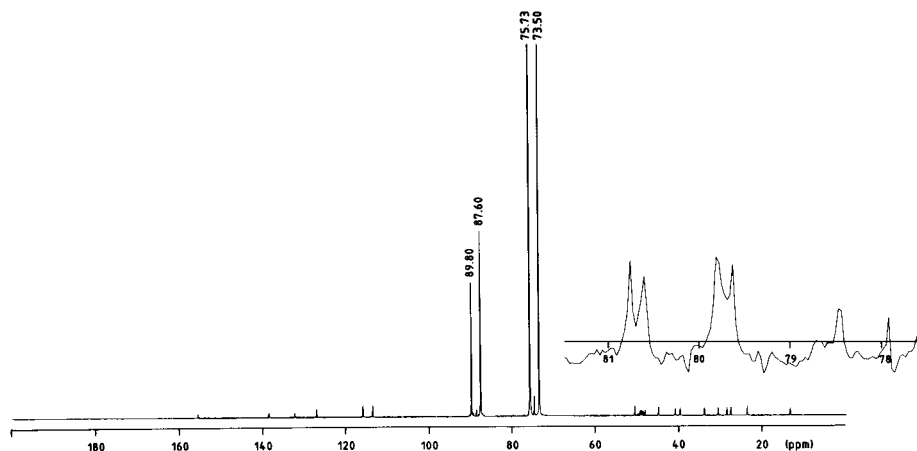


Fig . 3.  $^{13}\text{C}$ -NMR spectrum of  $^{13}\text{C}_2$ -EE<sub>2</sub> at 75.48MHz in  $\text{CD}_3\text{OD}$ .

The result of coupling between  $^{13}\text{C}_{17}$  and  $^{13}\text{C}_{19}$  and  $^{13}\text{C}_{20}$  around 80 ppm is shown in the insert.

and weak resonance at 155.5(s;  $\text{C}_3$ ), 138.7(s;  $\text{C}_5$ ); 132.6(s;  $\text{C}_{10}$ ); 127.1(d;  $\text{C}_1$ ); 116.1(d;  $\text{C}_4$ ); 113.7(d;  $\text{C}_2$ ); 80.2(dd,  $^1J(^{13}\text{C}_{17}-^{13}\text{C}_{19})=73\text{ Hz}$ ,  $^2J(^{13}\text{C}_{17}-^{13}\text{C}_{20})=12.3\text{ Hz}$ ;  $\text{C}_{17}$ ); 50.7(d;  $\text{C}_{14}$ ); 48.1(s;  $\text{C}_{13}$ ); 44.8(d;  $\text{C}_9$ ); 40.9(d;  $\text{C}_8$ ); 39.9(t;  $\text{C}_{16}$ ); 133.9(t;  $\text{C}_{12}$ ); 30.5(t;  $\text{C}_6$ ); 28.5(t;  $\text{C}_7$ ); 27.6(t;  $\text{C}_{11}$ ); 23.6(t;  $\text{C}_{15}$ ); 13.3(q;  $\text{C}_{18}$ ).

## CONCLUSION

From the characteristics of the steroid determined by IR, MS and NMR it can be concluded that indeed  $[19,20-^{13}\text{C}]\text{-EE}_2$  is synthesized.

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